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09/147,693

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Attorney Number:	1552	**U.S. Application S.N.:**	09/147,693
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Werner LUBITZ et al

Group Art Unit: 1636

Serial Number: 09/147,693

Examiner: Sandals, W.

Filed: February 17, 1999

Atty. Docket No. 100564-09005

For: NEW SYSTEMS FOR THE REGULATION OF GENE EXPRESSION

RESPONSE UNDER 37 C.F.R. '1.121

Commissioner for Patents
Washington, D.C. 20231

November 16, 2000

Sir:

This is a response to the Office Action dated August 16, 2000.

Claims 38-76 are currently pending.

Claims 38-41, 44-46, 50-53, 55-57, 60-62, 73, 75 and 76 are rejected under 35 USC §102(b) as being anticipated by Chen et al. (1990).

This rejection is respectfully traversed.

Applicants respectfully submit that Chen does not describe any operator sequences having different thermostability compared to a wild-type sequence with regard to binding a temperature-sensitive λ cI repressor. Rather, Chen describes a method of selecting mutations of the λ pL promoter, the transcriptional activity of which is lost entirely. Chen uses a DNA construct comprising the λ pL promoter, under the operative control of which

the *λ kil* gene is included as a suicide gene. Under repressed conditions (28 °C) the DNA construct is not transcribed due to the binding of the temperature-sensitive repressor cI857 to the oL sequences. When induced to 42 °C, the repressor is inactivated, the *kil* gene is expressed, and the cell is killed. In the case of Chen mutants are selected which nevertheless grow at 42 °C. Mutants have been found thereby having either deletions or base pair substitutions in the λ operator or promoter sequences, leading to the function of the promoter being generally lost, i.e., the promoter is no longer capable of effecting transcription of the *kil* gene, even if the repressor cI857 is absent. It is neither disclosed nor can it be assumed, that these mutations disclosed by Chen involve a change in the binding capacity of the λ cI857 repressor to the operator secuence. All we know is that, due to the mutation, RNA polymerase is no longer capable of transcribing the λ promoter. As to the deletion mutants, Chen states as follows on page 83, right column, 2nd paragraph: "It is therefore quite obvious that the lack of *λ kil* expression under restrictive conditions in these mutants was a direct result of promoter inactivation."

In the case of the mutants having base pair substitutions identified by Chen, in addition, a general decrease of transcriptional activity of the promoter is held responsible for the lack of expression of *λ kil* gene. On page 85, right column, 3rd paragraph the reference discloses as follows: "Each of these mutations reduces the correspondence with either the -35 or the -10 consensus sequence, and might therefore be expected to weaken the pL promoter." (Citation omitted.)

Contrary to Chen the present application describes changed λ operator sequences which influence the binding of the temperature-sensitive λ repressor and, thus, the temperature control of the expression system; however, these mutations do not affect the expression of the λ promoter itself. The constructs of the invention still effect the expression of the *ytic* gene after induction of the promoter to 42 °C, as can be seen from the Examples.

Contrary to the method of Chen, the selection procedure of the present invention is based on the total efficiency of the promoter at 42 °C. Chen selects promoter mutants which have lost their functionality, regardless of the function of the cI857 repressor. As the repressor is inactivated at 42 °C, the Chen mutants (based on deletions or base pair substitutions) still cannot form the *kil* gene product; thus, the non-occurrence of *kil* expression must have happened in a repressor-independent way (i.e., as a result of lost function of the promoter).

In sum, there is a basic difference between the mutations described by Chen and the mutations according to the invention. Whereas Chen aims at a loss of function of the promoter (inhibition of transcription catalyzed by RNA polymerase), the present invention is directed only to changed temperature control in the regulation of expression. Given these significant differences the rejection in view of Chen should not be maintained.

Claims 38-42, 44-48, 50-52, 66-70 and 73-76 are rejected under 35 USC. §103(a) as being unpatentable over Chen in view of Eliason et al., Pakula et al., Benson et al., U.S. Patent No. 4,634,678 and U.S. Patent No. 5,576,190.

The Examiner admits that Chen does not teach that the suicice gene included in the sequence is from PhiX174, nor that a mutator strain of bacteria may be used to induce mutations in the operator sequence, nor the specific temperature ranges of changes in the thermostability of the operator binding repressor, nor that the vector is a bacterial chromosomal vector, nor the use of multiple operator sequences. The Examiner relies on each of the cited references to make up for the deficiencies in Chen.

This rejection is respectfully traversed.

Chen has been discussed above. Since this reference does not teach what the Examiner relies on the reference as teaching, for this reason alone, the obviousness rejection should be withdrawn. Applicants have the following additional comments on the secondary references.

As applicants have previously indicated, Eliason does not disclose or suggest the method steps according to the invention. This reference discloses mutated λ operator sequences (see Figure 3) and tests the temperature sensitivity of their λ repressor binding (see Table 2). These data, however, show that the operator mutations disclosed in Eliason do not have an increased thermostability of the repressor binding, as compared to a wild-type sequence. Although Table 2 shows that the binding of the wild-type repressor (R_{cI236}) to the operator mutation OR1v3 is less sensitive to temperature than the wild-type operator

sequence (OR1$^+$), the binding at 37 °C is still nine times (50 mM KCl) or three times (200 mM KCl) less than that of the wild-type sequence. Ellason thus does not contain any indication of mutated operator sequences having an increased thermostability.

Pakula shows a mutated repressor which binds to wild-type OR regions differently depending upon the mutation. This has nothing to do with the present invention, which deals with a mutated OR region, not a mutated repressor. In addition, Pakula says nothing about thermostability.

While Benson discusses wild-type repressor binding to mutated OR sites, the reference does not discuss testing this binding over different temperatures in order to see whether the mutations have any effect on thermostability. Thus, this reference could not suggest the present invention.

As to the newly-cited '678 and '190 patents, applicants have the following additional comments.

The cloning and expression vectors given in the '678 patent contain λ pL, λ pR, or both promoters together. The changed promoter sequences mentioned by the Examiner are disclosed neither in the abstract nor in the summary. In the figures, however, a λ pL promoter can be found showing partial deletion of the operator sequences (celet on of oL3 in pSR72-N$^+$, pDH428, pDH438, cf. columns 31 and 32).

According to the '678 patent, this deletion is to result in reduced promoter strength; changed/reduced affinities of these sequences for the cI857 repressor are not mentioned. The reduced promoter strength is seen in the change of the -35 region of the promoter

(column 3, line 67 et seq.). The fact that λ oL and λ pR can be combined on a plasmid is neither novel nor imaginative, and it is to be assumed that the wild-type operator sequences oL and oR have different affinities for cI or cI857, since the operator sequences are not 100% identical. Changed thermostability of mutated oL/oR sequences compared with the respective wild-type sequences, however, cannot be gathered from this patent.

The plasmics disclosed in the '190 patent contain λ pL promoters which either show eliminated oL3 regions, or base pair substitution in the -10 region or the -10 region.

According to the '190 patent, base pair substitution in the -10 region leads to enhanced transcription, with repressor binding remaining constant (no changed affinity, see column 10, line 43 et seq.). The eliminated oL3 region is not further described with regard to its activity, and a further -10 mutant is said to have increased cI857 affinity, with transcriptional strength being unchanged. This mutation (in plasmid pHDM159) allegedly results in "tighter" regulation than the wild-type λ promoter (see column 7, lines 12-17). Any reference to increased or changed temperature stability of the binding of the temperature-sensitive λ cI857 repressor or pertinent experimental data cannot be found therein.

Since none of the cited references disclose or suggest the inventive features of the present invention, applicants respectfully submit that the rejection should be withdrawn.

Further rejections were made to dependent claims in view of the cited primary and secondary references, and further in view of various tertiary references. Applicants respectfully submit that none of these tertiary references would overcome the failure of the

more pertinent primary and secondary references to show the present invention. Therefore, these additional rejections should be withdrawn.

In the event this paper is not being timely filed, applicants respectfully petition for an appropriate extension of time. Any fees for such an extension together with any additional fees may be charged to Counsel's Deposit Account No. 01-2300.

Respectfully submitted,

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